The *Legionella pneumophila* effector Lpg1137 is a homologue of mitochondrial SLC25 carrier proteins, not of known serine proteases

Marcin Gradowski[1] and Krzysztof Pawłowski[1,2]

[1] Department of Experimental Design and Bioinformatics, Faculty of Agriculture and Biology, Warsaw University of Life Sciences, Warszawa, Poland

[2] Department of Translational Medicine, Clinical Sciences, Lund University, Lund, Sweden

ABSTRACT

Many bacterial effector proteins that are delivered to host cells during infection are enzymes targeting host cell signalling. Recently, *Legionella pneumophila* effector Lpg1137 was experimentally characterised as a serine protease that cleaves human syntaxin 17. We present strong bioinformatic evidence that Lpg1137 is a homologue of mitochondrial carrier proteins and is not related to known serine proteases. We also discuss how this finding can be reconciled with the apparently contradictory experimental results.

Subjects Bioinformatics, Molecular Biology

Keywords Structure prediction, Bacterial effectors, Proteases, Mitochondrial carriers

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Corresponding author
Krzysztof Pawłowski,
Krzysztof_Pawlowski@sggw.pl

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INTRODUCTION

Legionella pneumophila is an intracellular pathogen that causes a deadly respiratory infection called Legionnaire's disease. It typically infects amoebae, but can also enter human alveolar macrophages and proliferate within so-called Legionella-containing vacuoles (LCV) that are derived from the endoplasmic reticulum (*Eisenreich & Heuner, 2016*). To evade cellular defenses, for example to prevent the fusion of LCV with lysozymes, *L. pneumophila* and related species produce large repertoires of effectors that rewire host cell signalling (*Burstein et al., 2016*; *Isaac & Isberg, 2014*). A typical *L. pneumophila* strain produces approximately 300 different effectors that target processes as diverse as transcription and translation (*Rolando & Buchrieser, 2014*), and lipid (*Viner et al., 2012*), ubiquitin (*Zhou & Zhu, 2015*) and kinase signalling (*Haenssler & Isberg, 2011*). Many, if not the majority of bacterial effectors are distant homologues (*Alto & Orth, 2012*) or mimics of eukaryotic proteins (*Shi et al., 2016*).

The majority of *Legionella* effectors are experimentally uncharacterised, and a large fraction also remain unannotated despite large-scale bioinformatic endeavours. Such uncharacterised proteins evading function and structure prediction by automated bioinformatic pipelines can still be in many cases characterised *in silico* by careful application of diverse computational methods (*Pawlowski, 2008*). Effectors often turn out to be remote homologues of eukaryotic proteins, some harbouring well-known signalling domains, such as kinases (*Dong et al., 2016*) or proteases (*Liu et al., 2017*). Among the *Legionella* enzyme effectors, there are many cysteine proteases and metalloproteases, but very few serine proteases to date (*Burstein et al., 2016*).

Human Syntaxin 17 (Stx17) is a SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor) that localizes to endoplasmic reticulum (ER)-mitochondria contact sites. It performs diverse functions such as promoting mitochondrial fission and regulating ER Ca^{2+} homeostasis (*Arasaki et al., 2015*). Recently, it was reported that Stx17 is cleaved upon *L. pneumophila* infection, and that the cleavage depends on the presence of one of the multitude of as yet uncharacterised *L. pneumophila* effectors, Lpg1137(*Arasaki et al., 2017*). Further, this event "shuts down" communication between the ER and mitochondria.

Here, after an in-depth bioinformatic investigation, and unexpectedly for us, we can present strong bioinformatic evidence that Lpg1137 is actually a homologue of mitochondrial carrier proteins and is not related to known serine proteases.

METHODS

In order to explore possible distant sequence similarities of Lpg1137 to proteins of known structures, three established structural bioinformatic tools were used: FFAS03 (*Jaroszewski et al., 2011*), HHpred (*Hildebrand et al., 2009*) and Phyre2 (*Bennett-Lovsey et al., 2008*) with standard parameters and significance thresholds. The Phyre2 server was also used to build the three-dimensional structure model that was later visualized using Chimera software (*Yang et al., 2012*).

The multiple sequence alignment of mitochondrial carriers (MCs) and Lpg1137 homologues was built using the Muscle program (*Edgar, 2004*), and the sequence logos were created using the WebLogo server(*Crooks et al., 2004*).

For visual clustering of sequences, the CLANS algorithm (*Frickey & Lupas, 2004*) was applied to a set of representative sequences of MC pseudorepeats. The set was obtained by submitting three aligned MC pseudorepeats from the lncP protein (Refseq ID: WP_02722450, motifs defined by the Pfam database family Mito_carr, PF00153) (*Finn et al., 2016*) to two iterations of Jackhmmer search on the Uniprot database and by clustering with CD-HIT at a 35% sequence identity threshold (*Huang et al., 2010*). Then, the set was augmented by a set of homologues of Lpg1137 obtained from a Jackhmmer search (*Finn, Clements & Eddy, 2011*). CLANS was run with standard parameters using BLOSUM45 substitution matrix. For the graph, similarity relations with BLAST HSPs up to *E*-values of 1 were considered in order to visualize even distant similarities.

RESULTS AND DISCUSSION

Initially, the report by Arasaki and co-workers (*Arasaki et al., 2017*) of a novel effector serine protease prompted us to undertake sequence exploration with the expectation of finding more similar effector proteases. In a recent bioinformatic exploration of *Legionella* effectors, Lpg1137 homologues were found in 16 out of 41 species studied, making it a relatively widespread effector (*Burstein et al., 2016*). Although a Blast sequence search did

Table 1 Top structure predictions for Lpg1137.

Bioinformatic tool for structure prediction	Top hit: PDB code, name	Statistical significance for top hit	Region of Lpg1137 aligned to the hit	Sequence identity in the alignment
FFAS03	2lck, Mitochondrial uncoupling protein 2 [Mus musculus]	Z-score = −45.6	32 − 294	11%
HHpred	1okc, ADP, ATP carrier protein [Bos taurus]	E-value = 9.9e − 35	25 − 290	12%
Phyre2	4c9q, mitochondrial adp/atp carrier isoform 32 [yeast]	Confidence = 89.3	33 − 294	14%

not yield any obvious Lpg1137 homologues outside the *Legionella* and *Fluoribacter* genera, to our surprise, three independent bioinformatic tools for remote sequence similarity recognition (FFAS03, Phyre2, HHpred) indicated statistically significant similarity of Lpg1137 to mitochondrial carrier proteins (MCs, also known in mammals as solute carrier family 25, SLC25; see Table 1). The broad region of sequence similarity between Lpg1137 and the carrier proteins suggests it is likely that Lpg1137 forms a standard MC structure with a pseudo-threefold symmetry with six transmembrane helices (*Nury et al., 2006*; *Pebay-Peyroula et al., 2003*). The three sequence repeats, albeit not obvious to the eye, are visible upon inspection of an HHpred alignment to a MC structure (see Fig. 1). Sequence logos of the repeats in homologues of Lpg1137, compared to sequence logo of the eukaryotic mitochondrial carriers (See Figs. 2A, 2B respectively) support the structural similarity by highlighting the conservation of structurally important Pro and Gly residues (e.g., Pro at positions 15 and 239 in the logos or the YxG motif at positions 45–47). These prolines and glycines are among the most conserved residues among the MC proteins (*Wohlrab, 2005*). Since the logos were created from an unbiased common sequence alignment of the Lpg1137 homologues and the eukaryotic mitochondrial carriers, the conservation of these residues is noteworthy.

The finding of Lpg1137 similarity to mitochondrial carriers raises the obvious question: how can this be rationalized, given the convincing experimental data by Arasaki et al.? Actually, what these authors have shown is that the presence of the *L. pneumophila* Lpg1137 protein in transfected HeLa cells resulted in the cleavage of host syntaxin 17 (Stx17). They have also demonstrated that this cleavage is not observed when Ser68 of Lpg1137 (hypothesized to be the catalytic residue) is mutated to alanine or when a serine protease inhibitor is applied. However, the following scenario could be at play. Lpg1137, likely located in the mitochondrial inner membrane as reported by Arasaki et al. may activate an undisclosed serine protease, either directly, e.g., by physical interaction, or indirectly, e.g., by providing a required concentration of a small molecule it may be transporting, e.g., ATP. Allosteric activation of proteases is a known mechanism, described for cysteine proteases and serine proteases alike, and it may involve binding small molecules, dimerization, or binding of an accessory protein (*Arutyunova et al., 2014*; *De Regt et al., 2015*; *Lupardus et al., 2008*; *Zuhlsdorf et al., 2015*). Such a mode of activation would explain the dependence of Stx17 cleavage on the presence of Lpg1137 and on serine protease inhibitors. Alternatively, interaction with Lpg1137 may make syntaxin 17 prone to cleavage. The cleavage might be executed by an endogenous host protease or by an effector protease. However, experiments,

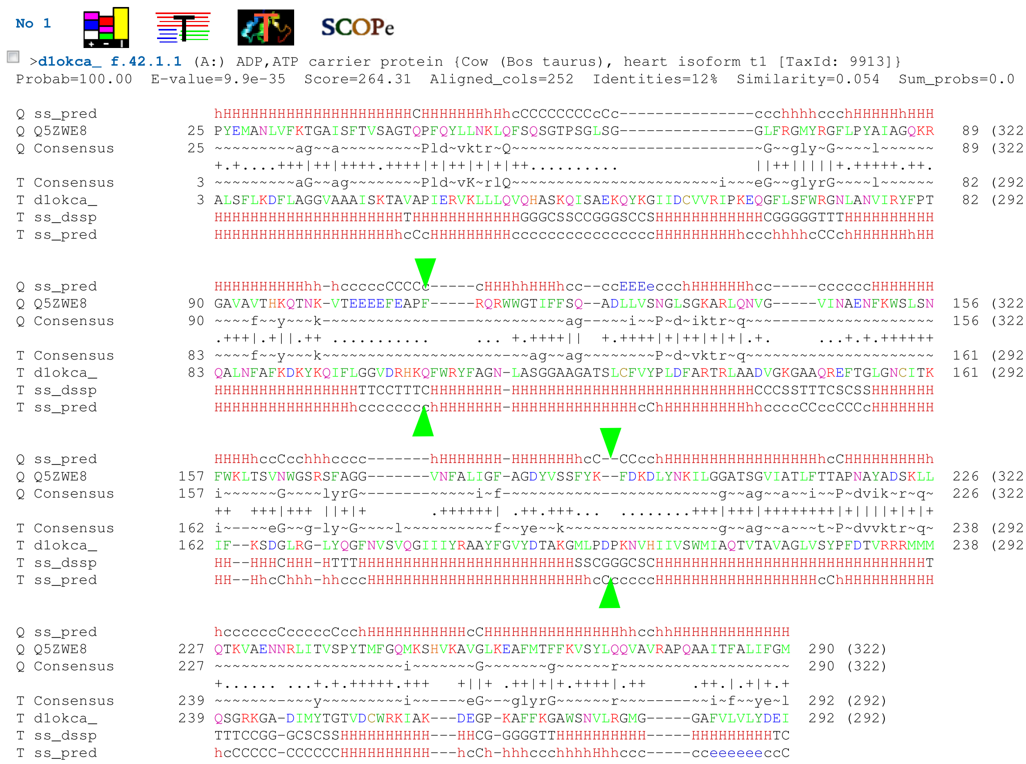

Figure 1 Protein sequence alignment between Lpg1137 (top, marked as Q5ZWE8) and bovine ADP, ATP carrier protein (bottom, marked as d1okca) obtained from the HHpred server. Predicted (ss_pred) and actual (ss_dssp) secondary structures shown: H-alpha helix, E-extended (beta strand), C-coil. Green triangles denote approximate borders of the three sequence repeats.

not bioinformatic predictions, should provide the definitive answer as to the functional identity of the Lpg1137 proteins.

Assuming the mitochondrial carrier prediction is correct, our bioinformatic analysis does not allow at this stage the prediction of detailed molecular function for Lpg1137. The known mitochondrial carriers exhibit great functional variability by transporting many diverse solutes (*Palmieri & Monne, 2016*). The sequence features of Lpg1137 and close homologs in the functionally important regions, e.g., the MC selectivity filter (*Nury et al., 2006*), do not permit it to be ascribed with confidence to any of the carrier subfamilies. Also, we cannot exclude that Lpg1137 might be a "pseudo-carrier", a carrier-like molecule lacking carrier activity.

Identification of Lpg1137's similarity to MCs allows the building of a model of its three-dimensional structure. Given the model, the special role of Ser68 can be addressed. In the structure model (see Fig. 3), this residue is located exposed in a loop region inside the mitochondrial matrix (or other organelle in whose membrane Lpg1137 might locate). Of note, S68 is not conserved among Lpg1137 close homologs, as would have been expected were it the catalytic residue (see Fig. 2). Thus, Ser68 might be mediating Lpg1137 interactions with other molecules or with Stx17 itself, which may be of importance for the downstream cleavage of Stx17.

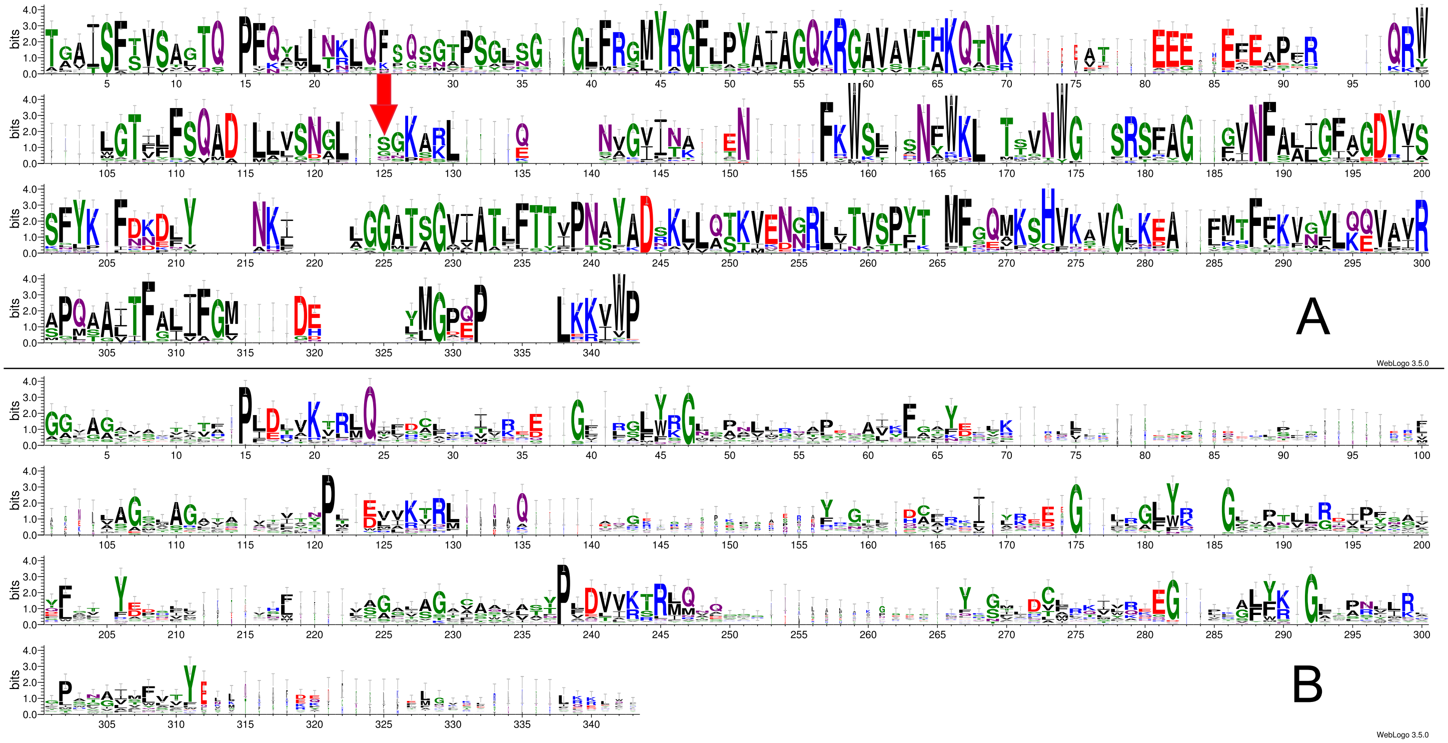

Figure 2 Sequence logos created from a combined Muscle alignment for Lpg1137 homologues (A) and human SLC mitochondrial carriers (B). Ser68 in Lpg1137 is located in column 125 of the sequence logo (red arrow). The Weblogo server was used (*Crooks et al., 2004*). The following residues are also shown in the structure model (Fig. 3): P15, G47, G175, P239 (numbering indicating positions in the sequence logo).

Based on our data, it appears less likely that Lpg1137 is itself a serine protease, as advocated by Arasaki et al. This would signify a very unique evolutionary appearance of a catalytic function on a carrier-like protein. Such a scenario appears to be supported by one experiment (*Arasaki et al., 2017*) (see Fig. 3E in the Arasaki et al. paper) which is interpreted as showing protease activity of recombinant Lpg1137. The Western blot does not show the appearance of lower molecular mass species of the cleaved protein. Also, this result is shown without replication and is not quantitative.

The sequence motif G-L-S-G-G around Ser68 in Lpg1137 is described by Arasaki et al. as the occurrence of a generic motif G-X-S-X-[GA] which bears superficial similarity to the partial catalytic signature of a serine protease active site. However, the rest of the classic serine protease catalytic triad (Ser-His-Asp) is not mentioned by those authors nor was it detected by us (*Arasaki et al., 2017*). According to the Merops database, serine proteases can be grouped in 12 clans divided into 36 families (*Rawlings, Barrett & Finn, 2016*). In one of the best studied families, trypsin, the active site serine is located in a conserved motif, G-[DNE]-S-G-[GS]-[PAST]. PrositeScan analysis (*Gattiker, Gasteiger & Bairoch, 2002*) indicates that the generic motif G-X-S-X-[GA] is non-specific and is very often found in randomised sequence databases (1,496 matches on a sample of 5,000 Swiss-Prot shuffled sequences). Thus, a motif that occurs in every third random sequence is unlikely to be a sign of a functional site. The precise motif surrounding Ser68 in Lpg1137 (G-L-S-G-G)

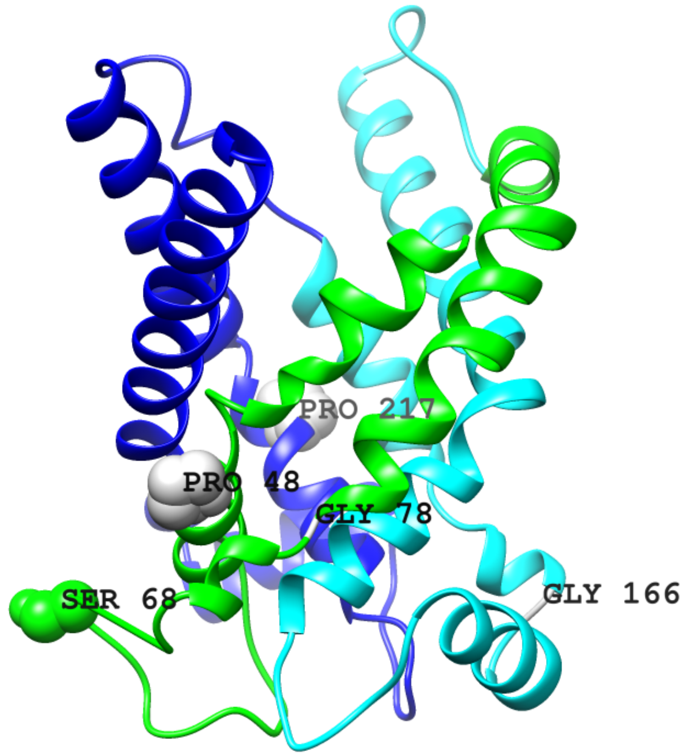

Figure 3 Three-dimensional structure model for Lpg1137. The three pseudorepeats indicated by color: green (1st), cyan (2nd) and dark blue (3rd). Serine 68 shown. Also, selected conserved residues shown: P48 (15), G78 (47), G166 (175), P217 (239), numbering in parentheses indicates positions in the sequence logo. Model obtained from the Phyre2 server using Protein Data Bank structure 4c9q (chain B) as template. Intermembrane space is on the top, mitochondrial matrix—on the bottom.

can be found in 1,235 sequences from the SwissProt database. However, only two of these are annotated as serine proteases. Therefore, the lack of recognizable His and Asp active site motifs and the poor specificity of the Ser68 motif make the similarity of Lpg1137 to known serine proteases doubtful.

When CLANS, the sequence similarity-based clustering algorithm, is applied to the set of Lpg1137 homologues and a representative set of MC repeats (see Fig. 4), it is obvious that the three sequence repeats of Lpg1137 are very distant from each other and from the rest of MC repeats. Indeed, in this analysis, all the eukaryotic MC repeats group in a single central cluster, while Lpg1137 repeats are located in distant outlier clusters.

Recently, another mitochondrial carrier *L. pneumophila* effector has been studied, lncP/LLO_1924 (*Dolezal et al., 2012*). This effector is somewhat less widespread in *Legionellas* than Lpg1137, and is found in 7 out of 41 genomes studied in the recent Burstein paper (*Burstein et al., 2016*). However, the lncP protein is only remotely similar to Lpg1137 (9% sequence identity in a FFAS03 sequence alignment with a significant Zscore equaling −41, see also Fig. 4). The role of lncP in infection is not clear; however, lncP can catalyze ATP efflux from mitochondria in infected cells (*Dolezal et al., 2012*).

Bacterial homologues of eukaryotic mitochondrial carriers are found in a handful of bacterial strains, usually infectious ones, including a few *Chlamydiales*, *Rickettsiales* and

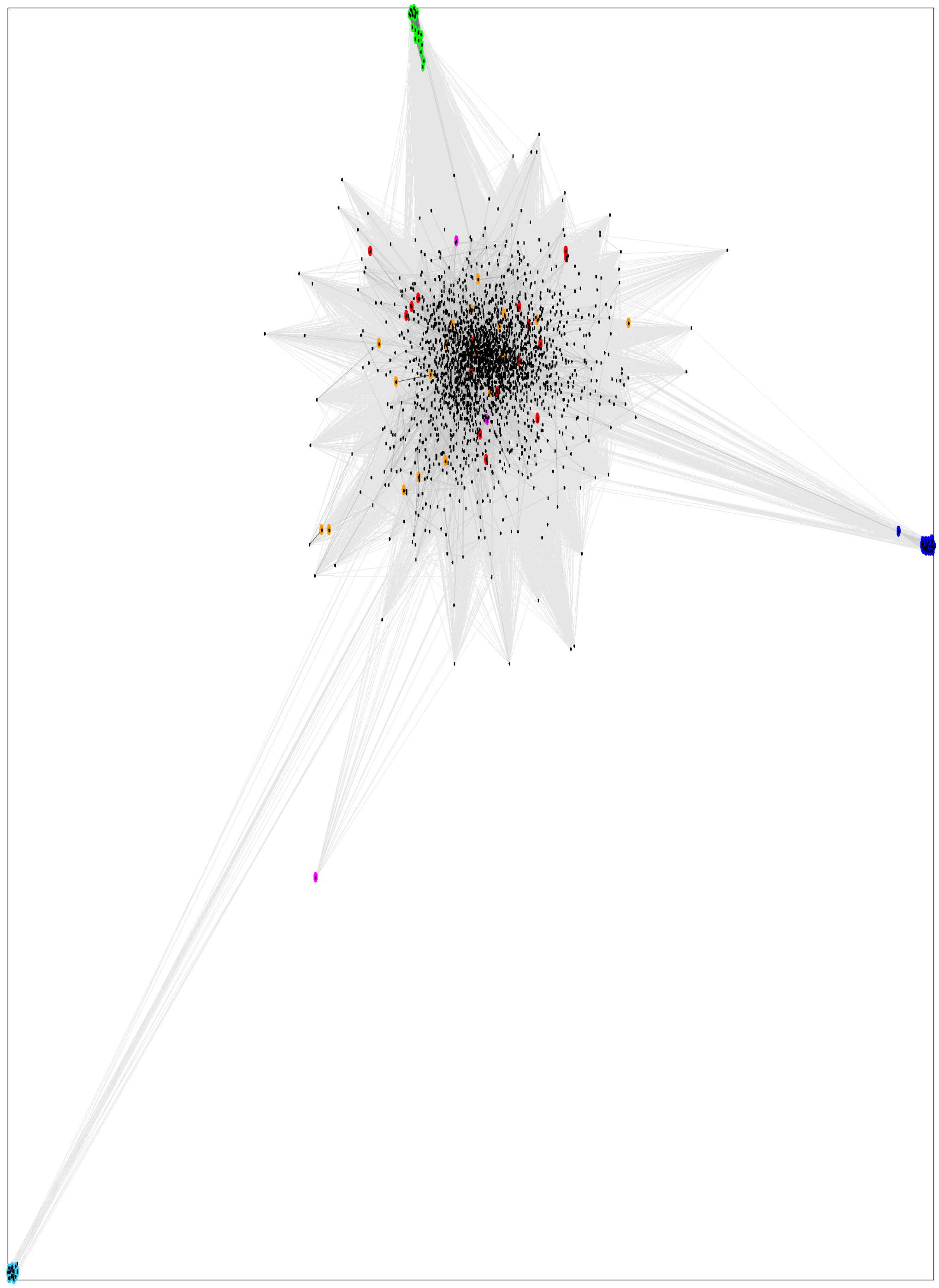

Figure 4 CLANS graph visualizing PSI-BLAST-detected sequence similarities between repeats of mitochondrial carrier proteins (Pfam: Mito_carr). The points represent sequences, and distances reflect the sequence dissimilarities. Black: eukaryotic repeats; red: viral repeats (Pfam); orange: bacterial repeats (Pfam); green: Lpg1137 homologues, 1st repeats; cyan: Lpg1137 homologues, 2nd repeats; dark blue: Lpg1137 homologues, 3rd repeats, magenta: *L. pneumophila* MC effector lncP (*Dolezal et al., 2012*).

Legionellales (Pfam family Mito_carr, PF00153). These proteins have been hypothesized to be the results of horizontal gene transfer from eukaryotes and to be involved in infection (*Dolezal et al., 2012*). Lpg1137 is only a very distant homologue of those annotated bacterial MC proteins and the eukaryotic MC proteins (see Fig. 4) with the middle sequence repeat being most divergent.

CONCLUSION

We present strong bioinformatic evidence that Lpg1137 is a mitochondrial carrier-like protein, a very distant homologue of SLC25 carriers. Nevertheless, current bioinformatic study does not constitute a proof that Lpg1137 is not a protease. What we present is strong evidence that Lpg1137 is not a homologue of known proteases, but rather a distant homologue of mitochondrial carriers. Building on the results of Arasaki et al., future experimental studies should include the solving of the three-dimensional structure of the protein and should cast light on its detailed function: be it proteolysis, small molecule transport across mitochondrial or other membrane, modulation of the activity of other MC proteins by oligomerization, or be it yet another role.

ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Marcin Gradowski performed the experiments, analyzed the data, prepared figures and/or tables, reviewed drafts of the paper.
- Krzysztof Pawłowski conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

Data Availability

The following information was supplied regarding data availability:

The raw data is supplied in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.3849#supplemental-information.

REFERENCES

Alto NM, Orth K. 2012. Subversion of cell signaling by pathogens. *Cold Spring Harbor Perspectives in Biology* **4**:a006114 DOI 10.1101/cshperspect.a006114.

Arasaki K, Mikami Y, Shames SR, Inoue H, Wakana Y, Tagaya M. 2017. Legionella effector Lpg1137 shuts down ER-mitochondria communication through cleavage of syntaxin 17. *Nature Communications* **8**:Article 15406 DOI 10.1038/ncomms15406.

Arasaki K, Shimizu H, Mogari H, Nishida N, Hirota N, Furuno A, Kudo Y, Baba M, Baba N, Cheng J, Fujimoto T, Ishihara N, Ortiz-Sandoval C, Barlow LD, Raturi A, Dohmae N, Wakana Y, Inoue H, Tani K, Dacks JB, Simmen T, Tagaya M. 2015. A role for the ancient SNARE syntaxin 17 in regulating mitochondrial division. *Developmental Cell* **32**:304–317 DOI 10.1016/j.devcel.2014.12.011.

Arutyunova E, Panwar P, Skiba PM, Gale N, Mak MW, Lemieux MJ. 2014. Allosteric regulation of rhomboid intramembrane proteolysis. *EMBO Journal* **33**:1869–1881 DOI 10.15252/embj.201488149.

Bennett-Lovsey RM, Herbert AD, Sternberg MJ, Kelley LA. 2008. Exploring the extremes of sequence/structure space with ensemble fold recognition in the program Phyre. *Proteins* **70**:611–625.

Burstein D, Amaro F, Zusman T, Lifshitz Z, Cohen O, Gilbert JA, Pupko T, Shuman HA, Segal G. 2016. Genomic analysis of 38 Legionella species identifies large and diverse effector repertoires. *Nature Genetics* **48**:167–175 DOI 10.1038/ng.3481.

Crooks GE, Hon G, Chandonia JM, Brenner SE. 2004. WebLogo: a sequence logo generator. *Genome Research* **14**:1188–1190 DOI 10.1101/gr.849004.

De Regt AK, Kim S, Sohn J, Grant RA, Baker TA, Sauer RT. 2015. A conserved activation cluster is required for allosteric communication in HtrA-family proteases. *Structure* **23**:517–526 DOI 10.1016/j.str.2015.01.012.

Dolezal P, Aili M, Tong J, Jiang JH, Marobbio CM, Lee SF, Schuelein R, Belluzzo S, Binova E, Mousnier A, Frankel G, Giannuzzi G, Palmieri F, Gabriel K, Naderer T, Hartland EL, Lithgow T. 2012. *Legionella pneumophila* secretes a mitochondrial carrier protein during infection. *PLOS Pathogens* **8**:e1002459 DOI 10.1371/journal.ppat.1002459.

Dong N, Niu M, Hu L, Yao Q, Zhou R, Shao F. 2016. Modulation of membrane phosphoinositide dynamics by the phosphatidylinositide 4-kinase activity of the Legionella LepB effector. *Nature Microbiology* **2**:Article 16236 DOI 10.1038/nmicrobiol.2016.236.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**:1792–1797 DOI 10.1093/nar/gkh340.

Eisenreich W, Heuner K. 2016. The life stage-specific pathometabolism of *Legionella pneumophila*. *FEBS Letters* **590**:3868–3886 DOI 10.1002/1873-3468.12326.

Finn RD, Clements J, Eddy SR. 2011. HMMER web server: interactive sequence similarity searching. *Nucleic Acids Research* **39**:W29–W37 DOI 10.1093/nar/gkr367.

Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, Potter SC, Punta M, Qureshi M, Sangrador-Vegas A, Salazar GA, Tate J, Bateman A. 2016. The Pfam protein families database: towards a more sustainable future. *Nucleic Acids Research* **44**:D279–D285 DOI 10.1093/nar/gkv1344.

Frickey T, Lupas A. 2004. CLANS: a Java application for visualizing protein families based on pairwise similarity. *Bioinformatics* **20**:3702–3704 DOI 10.1093/bioinformatics/bth444.

Gattiker A, Gasteiger E, Bairoch A. 2002. ScanProsite: a reference implementation of a PROSITE scanning tool. *Applied Bioinformatics* **1**:107–108.

Haenssler E, Isberg RR. 2011. Control of host cell phosphorylation by *Legionella pneumophila*. *Frontiers in Microbiology* **2**:Article 64 DOI 10.3389/fmicb.2011.00064.

Hildebrand A, Remmert M, Biegert A, Soding J. 2009. Fast and accurate automatic structure prediction with HHpred. *Proteins* **77(Suppl 9)**:128–132 DOI 10.1002/prot.22499.

Huang Y, Niu B, Gao Y, Fu L, Li W. 2010. CD-HIT Suite: a web server for clustering and comparing biological sequences. *Bioinformatics* **26**:680–682 DOI 10.1093/bioinformatics/btq003.

Isaac DT, Isberg R. 2014. Master manipulators: an update on *Legionella pneumophila* Icm/Dot translocated substrates and their host targets. *Future Microbiology* **9**:343–359 DOI 10.2217/fmb.13.162.

Jaroszewski L, Li Z, Cai XH, Weber C, Godzik A. 2011. FFAS server: novel features and applications. *Nucleic Acids Research* **39(Suppl 2)**:W38–W44 DOI 10.1093/nar/gkr441.

Liu Y, Zhu W, Tan Y, Nakayasu ES, Staiger CJ, Luo ZQ. 2017. A legionella effector disrupts host cytoskeletal structure by cleaving actin. *PLOS Pathogens* **13**:e1006186 DOI 10.1371/journal.ppat.1006186.

Lupardus PJ, Shen A, Bogyo M, Garcia KC. 2008. Small molecule-induced allosteric activation of the Vibrio cholerae RTX cysteine protease domain. *Science* **322**:265–268 DOI 10.1126/science.1162403.

Nury H, Dahout-Gonzalez C, Trezeguet V, Lauquin GJ, Brandolin G, Pebay-Peyroula E. 2006. Relations between structure and function of the mitochondrial ADP/ATP carrier. *Annual Review of Biochemistry* **75**:713–741 DOI 10.1146/annurev.biochem.75.103004.142747.

Palmieri F, Monne M. 2016. Discoveries, metabolic roles and diseases of mitochondrial carriers: a review. *Biochimica et Biophysica Acta/General Subjects* **1863**:2362–2378 DOI 10.1016/j.bbamcr.2016.03.007.

Pawlowski K. 2008. Uncharacterized/hypothetical proteins in biomedical 'omics' experiments: is novelty being swept under the carpet? *Briefings in Functional Genomics & Proteomics* **7**:283–290 DOI 10.1093/bfgp/eln033.

Pebay-Peyroula E, Dahout-Gonzalez C, Kahn R, Trezeguet V, Lauquin GJ, Brandolin G. 2003. Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside. *Nature* **426**:39–44 DOI 10.1038/nature02056.

Rawlings ND, Barrett AJ, Finn R. 2016. Twenty years of the MEROPS database of proteolytic enzymes, their substrates and inhibitors. *Nucleic Acids Research* **44**:D343–D350 DOI 10.1093/nar/gkv1118.

Rolando M, Buchrieser C. 2014. *Legionella pneumophila* type IV effectors hijack the transcription and translation machinery of the host cell. *Trends in Cell Biology* **24**:771–778 DOI 10.1016/j.tcb.2014.06.002.

Shi X, Halder P, Yavuz H, Jahn R, Shuman HA. 2016. Direct targeting of membrane fusion by SNARE mimicry: convergent evolution of legionella effectors. *Proceedings of the National Academy of Sciences of the United States of America* **113**:8807–8812 DOI 10.1073/pnas.1608755113.

Viner R, Chetrit D, Ehrlich M, Segal G. 2012. Identification of two *Legionella pneumophila* effectors that manipulate host phospholipids biosynthesis. *PLOS Pathogens* **8**:e1002988 DOI 10.1371/journal.ppat.1002988.

Wohlrab H. 2005. The human mitochondrial transport protein family: identification and protein regions significant for transport function and substrate specificity. *Biochimica et Biophysica Acta/General Subjects* **1709**:157–168 DOI 10.1016/j.bbabio.2005.07.003.

Yang Z, Lasker K, Schneidman-Duhovny D, Webb B, Huang CC, Pettersen EF, Goddard TD, Meng EC, Sali A, Ferrin TE. 2012. UCSF Chimera, MODELLER, and IMP: an integrated modeling system. *Journal of Structural Biology* **179**:269–278 DOI 10.1016/j.jsb.2011.09.006.

Zhou Y, Zhu Y. 2015. Diversity of bacterial manipulation of the host ubiquitin pathways. *Cellular Microbiology* **17**:26–34 DOI 10.1111/cmi.12384.

Zuhlsdorf M, Werten S, Klupp BG, Palm GJ, Mettenleiter TC, Hinrichs W. 2015. Dimerization-induced allosteric changes of the oxyanion-hole loop activate the pseudorabies virus assemblin pUL26N, a herpesvirus serine protease. *PLOS Pathogens* **11**:e1005045 DOI 10.1371/journal.ppat.1005045.